Wyoming Game & Fish Department Standard Operating Procedure or Policy (SOP)			
SOP Title: Procedures for Isolating *Mycoplasma ovipneumoniae*			
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Area: WGFD	**Date**:1-26-2012	**Rev Date**: 1/7/2016	**Rev Level**: 4

Wyoming Game & Fish Department
Wildlife Disease Laboratory

Purpose and application of procedure:

To isolate *Mycoplasma ovipneumoniae*.

Procedure:
First read the Laboratory Common Sense SOP WGF.18, Sample Handling SOP WGF.17; then continue the protocol
Record all QC information on QC coversheet

Materials

1. Disposable gloves
2. Protective glasses
3. Lab coats
4. Modified TSB-1 broth
5. 5ml snap cap tubes
6. Pipette-gun
7. 5ml serological pipettes
8. Scissors
9. Columbia Blood Agar plates (Hardy Diagnostics A16)
10. 200µl pipette designated for Mycoplasma ovipneumoniae
11. 100 µl pipette tips
12. 6 inch polyester applicators
13. Loop
14. Bacti-incinerator
15. CO_2 incubator

Safety

1. Lab personnel must wear protective coat, gloves and glasses at all times.
2. Setting up plates should occur in biological hood, reading of plates does not have to occur in biological hood.
3. For specific safety information regarding the reagents involved in this test refer to the MSDS binder on the second shelf inside the door of the wildlife disease laboratory housed in the WSVL room 1104.
4. Avoid any contact of the modified TSB-1 broth to skin and mucous membranes.

Test Preparation

1. Samples should arrive in form of polyester swabs inside of BD Port-a-Culs, or fresh tissue sample.
2. When sample specimen is received assign it a WSVL accession number, or GF number and place respective numbers on the specimen vial and paperwork. Specimens are kept at 4^{o}C until they processed.
3. Samples should be processed the same day that they arrive in the lab.
4. Snap cap tubes will be labeled to match animal ID's on the sample.
5. 2mls of modified TSB-1 will be put into each of the snap cap tubes.
6. Swabs will be removed aseptically from the BD Port-a-Culs with scissors or forceps.
7. The swab tip will be placed into the snap cap tube and the shaft will be cut off to allow the lid to be placed back on the tube.
8. The lid of the snap cap will be left on loose.
9. Tissue samples will be stomached in mod TSB-1. Aseptically remove a piece of tissue (at least 0.5" x 0.5", if possible) and place in stomacher bag with 2ml modified TSB-1 broth. After bag is stomached, cut corner of bag and pour contents into 5m; snap cap tube.
10. A snap cap tube of 1mL of modified TSB-1 will also be incubated to serve as a blank for a PCR control.
11. The snap cap tubes (with broth and swab, or broth and tissue) will be placed into a rack, in the CO_2 incubator at 37^{o}C and 10% CO_2.
12. Samples in broth will incubate for 48hrs.
13. Samples should be checked at ~18-24 hours for color change. If broth has changed from red to yellow, samples should be plated and pulled for PCR immediately. Those remaining red should be incubated until 48 hours.
14. At 48hrs, the snap cap tubes are removed from the incubator.
15. At this time, 1mL of broth will be removed for PCR (see WGF.19 Mycoplasma PCR_rev.1).
16. Columbia blood agar plates should be labeled with the animal ID's from the broth snap cap tubes.
17. 100µls of broth taken from the snap cap tubes will be aspirated onto one edge of the Columbia blood agar plate.
18. These drops will sit ~30 seconds on plate, then will be smeared across that half of the plate with a polyester swab that has been soaked in the corresponding sample broth.
19. Using the loop and bacti-incinerator, the second half of the plate will be streaked for isolation.
20. The plate will then be returned to the CO_2 incubator at 37^{o}C and 10% CO_2.
21. The plate will be read once a day for up to five days.
22. Once broth is plated, broth can be put into -70 freezer until PCR is done, in case the broth needs to be resampled.

Preparation of Reagents

Warning – Be extrememly careful when working with thalium acetate, very toxic.

1 Liter Batch of Modified TSB-1

Amount	Component
30g	Tryptone Soya Broth
10g	D-Lactose Monohydrate
200mL	Porcine Serum – heat inactivated (Rocky Mountain Biologicals)
7.25mg	Amphotericin B (dissolved in deionized H_20-pH 11)
1,323,661units	Penicillin G Potassium Salt
23.27mL	Thallium Acetate (10mg/mL(de-oinized H_20)
18mg	Phenol Red
1000mL	Distilled H_20

Identification

1. *Mycoplasma ovipneumoniae* will present as hemolytic colonies that are growing in the media. There will not be any visible bacterial colonies growing on top of the media. Hemolytic colonies with visible colonies on top of the media may be *Mycoplasma arginini* or a mixed culture of *Mycoplasma ovipneumoniae* and *Mycoplasma arginini*.
2. See attached photos at end of SOP.

Associated SOPs:
WGF19. Mycoplasma PCR_rev.1

Associated Forms or Files:

Bact worksheet_Form 38_Mycoplasma

References:

Revision History: Rev 1. Added 'heat-inactivated' to porcine serum.
Rev 2. Updated preparation of reagents with more accurate names and formulas. Updated tissue protocol, and adding blank. Photo of *M. ovi*.
Rev. 3 Updated tissue protocol
Rev. 4 Updated amount of broth for PCR from 200µl to 1ml

Quality Control:

Freshly prepared modified TSB-1 broth can be stored at 2-7°C, for 3 months.

If contamination is presenting consistently on plates, the modified TSB-1 broth will need to be remade.

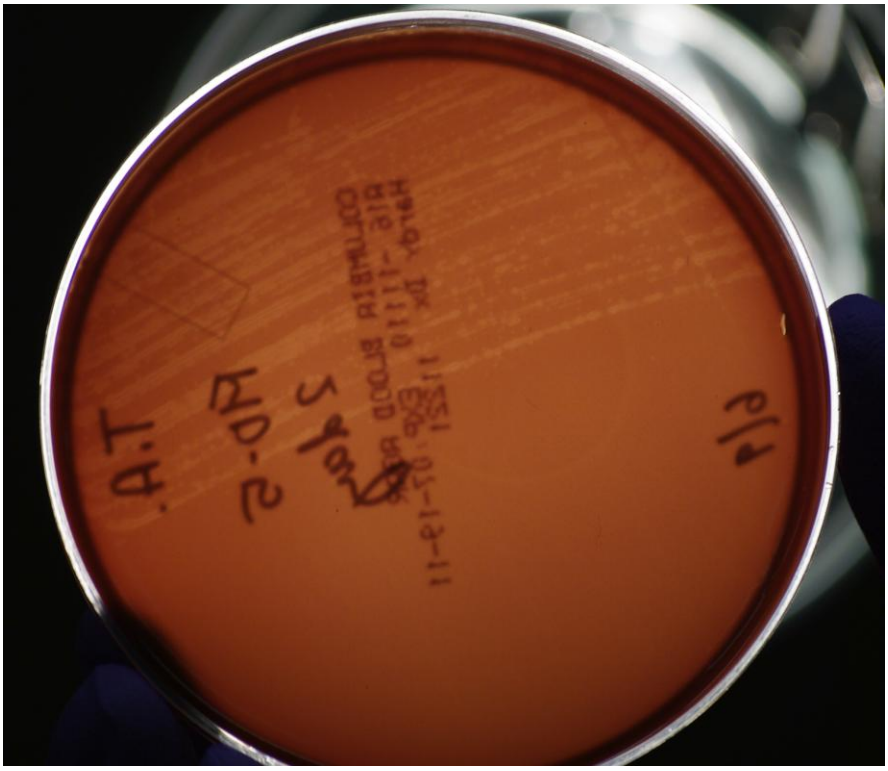
Samples should be processed the same day they arrive in the lab.

All samples will be plated for culture and ran on PCR.

Waste Disposal:
See also Waste Disposal SOP WGF.16

1. Once samples are deemed ready for disposal, broth is thawed out and poured into waste container stored under sink on North wall of lab.
2. Swabs and empty snap cap tubes can be discarded in biohazard trash.
3. Once waste container is full, University of Wyoming Environmental Health and Safety Waste Management department should be contacted to pick up.

Distribution List: Hank Edwards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